

Custom primer design

OligoPerfect Primer Designer demo guide

Introduction

Invitrogen™ OligoPerfect™ Primer Designer is a comprehensive online tool intended to simplify the process of designing DNA primers for PCR, sequencing by capillary electrophoresis (CE), or cloning. This tool takes into account multiple parameters such as GC content, melting temperature (T_m), primer length, and sequence specificity, which are crucial for the successful design of oligonucleotide primers.

Getting started

To begin, choose the intended application to access specialized features for the design:

- **Endpoint PCR**—allows you to specify the analysis region and amplicon size
- **CE sequencing**—allows you to define the sequencing region and the spacing between sequencing primers within that region
- **Cloning**—allows you to add 5′ modifications such as restriction sites and proteolytic cleavage sites to your primers, as well as modifications for specific cloning technologies such as Invitrogen™ Gateway™ and Directional TOPO™ cloning



All applications use a three-step process—design, select, and review. In the design step, you will be asked to enter project details and the template sequence(s) you are targeting. The select step displays several candidate primers along with a visual layout of primer location along the entered sequence. Primers or primer pairs can be selected at this step and added to the review screen. The review step provides basic information about the primers, including sequence, length, targeted strand, GC content, T_m , and price. Primers or whole projects can be selected and added to the cart from the review screen.

Designing primers



Endpoint PCR

Design

1. Enter project name, researcher name, and any notes you would like to retain with your project.
2. Enter the target sequence in FASTA format, or drag and drop a FASTA file into the sequence box. An example of this format is given in the sequence window. Up to 50 sequences can be entered at one time. Each sequence should be 10 kb or less.
3. Click “Show Parameters” to review or change desired output settings, primer selection criteria (length, T_m , GC content), and reaction conditions. A custom region can be targeted by specifying the included and excluded regions and the product size range. After updating parameters, select “Apply Changes”.
4. Click “Save and Continue”.

Select

1. Click on a sequence name from the sequence list to view candidate primers. A visual representation of your target sequence and the associated primers will appear at the top of the right panel. Primer pairs will be highlighted when the cursor hovers over a primer pair in the list below. Clicking on the sequences in the primer list will switch to the sequence viewer.
2. Select desired primers using the tick boxes in the primer list, and click “Add to review”.
3. Repeat for any additional sequences in the sequence list.
4. Once all primers have been selected, click the “Review & Order” button in the upper-right corner.

Review

1. Expand projects using the “v” icon to see individual oligo details.
2. Select desired primers using the tick boxes for individual primers or whole projects, and click “Add to Cart”. Projects and primers not added to the cart will be retained on the review screen until you are ready to order.
3. You will be redirected to your thermofisher.com cart where you can complete your order.



CE sequencing

Design

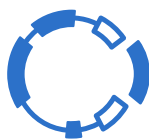
1. Enter project name, researcher name, and any notes you would like to retain with your project.
2. Enter the target sequence in FASTA format, or drag and drop a FASTA file into the sequence box. An example of this format is given in the sequence window. Up to 50 sequences can be entered at one time. Each sequence should be 10 kb or less.
3. Click “Show Parameters” to review or change desired output settings, primer selection criteria (length, T_m , GC content), and reaction conditions. A custom region can be targeted by specifying the sequencing region and product size range. Primer spacing can also be defined here. After updating parameters, select “Apply Changes”.
4. Click “Save and Continue”.

Select

1. Click on a sequence name from the sequence list to view candidate primers. A visual representation of your target sequence and the associated primers will appear at the top of the right panel. Primers will be highlighted when the cursor hovers over a primer in the list below. Clicking on the sequences in the primer list will switch to the sequence viewer.
2. Select all or individual primers using the tick boxes in the primer list, and click “Add to review”.
3. Repeat for any additional sequences in the sequence list.
4. Once all primers have been selected, click the “Review & Order” button in the upper-right corner.

Review

1. Expand projects using the “v” icon to see individual oligo details.
2. Select desired primers using the tick boxes for individual primers or whole projects, and click “Add to Cart”. Projects and primers not added to the cart will be retained on the review screen until you are ready to order.
3. You will be redirected to your thermofisher.com cart where you can complete your order.



Cloning

Design

1. Enter project name, researcher name, and any notes you would like to retain with your project.
2. Enter a target sequence of 10 kb or less in FASTA format, or drag and drop a FASTA file into the sequence box. An example of this format is given in the sequence window.
3. Indicate the region of your sequence that should be included in the amplicon by filling in the “Included Region” box.
4. Click “Show Parameters” to review or change desired output settings, primer selection criteria (length, T_m , GC content), and reaction conditions. Additional sequences, such as proteolytic cleavage sites or cloning sites, can be added to the 5' ends of the primers. After updating parameters, select “Apply Changes”.
5. Click “Save and Continue”.

Select

1. Click on a sequence name from the sequence list to view candidate primers. A visual representation of your target sequence and the associated primers will appear at the top of the right panel. Primers will be highlighted when the cursor hovers over a primer in the list below. Clicking on the sequences in the primer list will switch to the sequence viewer.
Note: Due to limited flexibility in primer sequence for a specific defined cloning region, you may encounter a “no primers found” error. If this happens, click back to the “Design” screen using the left navigation and then click “Show Parameters” to adjust the design parameters to be more flexible. Here you may also use the “Pick primer anyway” tick box to show candidate primers even if they do not meet your preferred criteria.
2. Select all or individual primers using the tick boxes in the primer list, and click “Add to review”.
3. Once all primers have been selected, click the “Review & Order” button in the upper-right corner.

Review

1. Expand projects using the “v” icon to see individual oligo details.
Note: If you have elected to include Gateway cloning sites in your primer sequences, there will be multiple ambiguous bases included in the primer design indicated by a “Y” notation. The purpose of these bases is to maintain the reading frame. If the primers are ordered as-is, each “Y” position of the oligos will contain a mix of C and T bases. If you wish to define a specific codon at these positions, please copy and paste the oligos into our [Custom Standard DNA Oligos ordering tool](#) and make the sequence adjustments there rather than adding them directly to your cart in the OligoPerfect Primer Designer.
2. Select desired primers using the tick boxes for individual primers or whole projects, and click “Add to Cart”. Projects and primers not added to the cart will be retained on your account until you are ready to order.
3. You will be redirected to your thermofisher.com cart where you can complete your order.